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Original Research

Housing Conditions Modulate the Severity of *Mycoplasma pulmonis* Infection in Mice Deficient in Class A Scavenger Receptor

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Mycoplasmosis is a frequent causative microbial agent of community-acquired pneumonia and has been linked to exacerbation of chronic obstructive pulmonary disease. The macrophage class A scavenger receptor (SRA) facilitates the clearance of noxious particles, oxidants, and infectious organisms by alveolar macrophages. We examined wildtype and SRA$^{-/-}$ mice, housed in either individually ventilated or static filter-top cages that were cycled with fresh bedding every 14 d, as a model of gene–environment interaction on the outcome of pulmonary *Mycoplasma pulmonis* infection. Intracage NH_3 gas measurements were recorded daily prior to infection. Mice were intranasally infected with 1×10^7 cfu *M. pulmonis* UAB CT and evaluated at 3, 7, and 14 d after inoculation. Wildtype mice cleared 99.5% of pulmonary *M. pulmonis* by 3 d after infection but remained chronically infected through the study. SRA$^{-/-}$ mice were chronically infected with 40-fold higher mycoplasma numbers than were wildtype mice. *M. pulmonis* caused a chronic mixed inflammatory response that was accompanied with high levels of IL1β, KC, MCP1, and TNFα in SRA$^{-/-}$ mice, whereas pulmonary inflammation in WT mice was represented by a monocytosis with elevation of IL1β. Housing had a prominent influence on the severity and persistence of mycoplasmosis in SRA$^{-/-}$ mice. SRA$^{-/-}$ mice housed in static cages had an improved recovery and significant changes in surfactant proteins SPA and SPD compared with baseline levels. These results indicate that SRA is required to prevent chronic mycoplasma infection of the lung. Furthermore, environmental conditions may exacerbate chronic inflammation in *M. pulmonis*-infected SRA$^{-/-}$ mice.

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; KC, keratinocyte-derived chemokine (*CXCL1*); MCP1, monocyte chemotactic protein 1; SPA, surfactant protein A (*SFTPA1*); SPB, surfactant protein B (*SFTPB*); SPD, surfactant protein D (*SFTPD*); SRA, class A scavenger receptor (*MSR1*); WT, wildtype.

There are numerous options for the housing and husbandry of rodents in the laboratory setting. Various available choices in caging, bedding material, and cage-change frequency have the potential to effect physiologic values and thus experimental outcomes.[20,108] In many facilities, current practices involve performing cage changes every 1 to 2 wk, with some facilities exploring the possibility of extending these practices to every 4 wk.[97] Cage-change frequency practices are established at various institutions after consideration of several variables that affect animal health, welfare, and cost. Ideally, an appropriate sanitation program provides clean and dry bedding, adequate air quality, and clean cage surfaces and accessories.[44] When establishing performance standards for a sanitation program that are different from those which are recommended in the *Guide for the Care and Use of Animals in Research*,[44] microenvironmental conditions, including intracage humidity, temperature, animal behavior and appearance, microbiologic loads, and levels of pollutants such as CO_2 and NH_3, should be evaluated and verified. Although there are currently no established NH_3 exposure limits for laboratory animals, the human occupational exposure limit of 25 ppm as an 8-h time-weighted average, established by the National Institute for Occupational Safety and Health, is often referenced as a guideline for animals.[95] Multiple factors, such as animal cage density, sex, age, bedding type, reusable compared with disposable caging, static caging compared with IVC, and cage-change frequency, influence intracage and ambient NH_3 levels.[82,83,97] Only limited information is available that addresses the effect of natural intracage NH_3 levels on respiratory function in experimental rodents and whether exposure to high NH_3 levels under current standard practices affects the results of respiratory disease research.

Ammonia is an alkaline, corrosive, and irritant gas that is very water soluble. It reacts with the moisture of the mucous membranes of the eyes, mouth, and respiratory tract to form ammonium hydroxide in an exothermic reaction, resulting in thermal and chemical burns.[68] Clinical symptoms in humans exposed to high levels of NH_3 include eye irritation, headaches, and multiple acute and chronic respiratory symptoms, such as irritation of the

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nose, pharynx, and sinuses, and in severe cases, development of bronchitis and hyper-reactive airway disease.[79] Animals are similarly susceptible to NH_3-induced pulmonary disease.[23,31,48]

Mice exposed to naturally increasing levels of intracage NH_3 can develop lesions in the rostral nasal cavity, with decreasing severity of the lesions moving caudally into the nasopharynx, and no lesions in the lung.[97] However, dust is another common environmental pollutant that is often present in animal settings. Dust particles readily absorb NH_3, which then serve as a source of NH_3 deposition into the lower respiratory tract. Dust particulate can range from large (300 µm), minimally respirable particles to very fine (< 50 µm) particulate matter, which can settle deep within the alveoli.[10,102] The mucociliary system of the respiratory tract is the first line of defense against inspired noxious stimuli and pathogens. Exposure of the ciliated respiratory epithelium to the damaging effects of NH_3 are known to cause decreased mucociliary beating.[56] Disruption of the respiratory mucociliary escalator initiated by NH_3 exposure can then promote establishment of chronic infections and inflammation of the airway mucosa.[11,87] Therefore, NH_3 potentially can cause pathophysiologic changes of the lung in the absence of histopathologic lesions.

Our primary goal was to analyze the effect of 2 housing modalities, which result in different intracage NH_3 concentrations, on mice that were challenged with a respiratory pathogen. *Mycoplasma pulmonis* was chosen as a model because it is a well-established model in rodents which causes chronic mycoplasmosis and reproduces the features of *M. pneumoniae* in humans.[22,41] *M. pneumoniae* infection is a frequent and contagious etiology of community-acquired pneumonia causing tracheobronchitis, sneezing, cough, and inflammation of the respiratory tract.[8,12,47,63] Moreover, atypical and difficult-to-detect respiratory pathogens such as *Chlamydophila pneumoniae* and *Mycoplasma pneumonia*e that can establish chronic asymptomatic infections may contribute to both the development and exacerbation of COPD[26,45,57,58,62,63,66,72,96,103] and asthma.[8,51,65] Infection with *M. pulmonis* in rodents causes rhinitis, otitis media, tracheitis, and pneumonia, which can be exacerbated by housing conditions and genetic background.[14,32,85] The mechanism of pathogenicity of mycoplasmas continues to be an area of interest in the research.

The innate host factors protecting against pulmonary mycoplasmosis include the secreted surfactant protein opsonins SPA and SPD, surfactant phospholipids, and the molecular pattern-recognition receptor TLR2.[15,16,54,74] Therefore, compared with their wildtype (WT) counterparts, SPA-deficient mice infected with either *M. pulmonis* or *M. pneumoniae* develop more severe inflammation and have decreased capacity to clear these infections from the lungs.[43] In addition, TLR2-deficient mice exhibit decreased clearance and increased inflammation in response to mycoplasma infection.[60,104]

Second, we wanted to study the effects of SRA deficiency in mycoplasmosis. The class A scavenger receptor (SRA) modulates inflammatory responses and mediates the clearance of airborne oxidants, particulates, and respiratory pathogens.[3,17,18,49,88,101] Inhibition of SRA expression in alveolar macrophages in an elastase–LPS model of COPD was associated with decreased clearance of *Haemophilus influenzae*.[33] Lack of SRA similarly impaired alveolar macrophage-mediated clearance of *Streptococcus pneumoniae*,[5] environmental particles,[6] and ozone-oxidized lipids[18] by alveolar macrophages. Absence of SRA also enhanced hyperoxia-induced lung injury[49] and exacerbated inflammation in response to *Staphylococcus aureus* infection.[88] SRA appears to have antiinflammatory properties with the capacity to modify macrophage phenotype and suppress polarization toward the M1 alternative macrophage activation state.[13] The SRA gene (*MSR1*) is polymorphic in both mice and humans.[19,29,105] Genetic association studies in humans, however, showed that subjects with truncations or point mutations in *MSR1* have significantly increased risk for the development of pulmonary diseases such as COPD[33,38,71,94] and asthma.[5] Our understanding of the immune factors that contribute to mycoplasmosis is far from complete.

In the present study, by investigating the role of SRA in mycoplasmosis jointly with the effects of housing, we demonstrated that genetic and environmental factors both serve as critical players in disease progression. We show that SRA-deficient mice are susceptible to chronic colonization with *M. pulmonis* and development of chronic mycoplasma-induced bronchopneumonia characterized by persistent multicellular inflammation. Furthermore, we show that housing conditions influence the effect of SRA deficiency on the severity of mycoplasmosis. Taken together, these results indicate that lack of SRA function impairs host protection against both infectious and environmental insults.

Materials and Methods

Mice. All experimental protocols were approved by the Pennsylvania State University College of Medicine Animal Care and Use Committee and adhered to the *Guide*.[44] Experiments involved 56 female C57BL/6J (WT) mice, acquired at 6 to 7 wk of age (catalog no. 000664, Jackson Laboratories, Bar Harbor, ME). Two sets of 8-wk-old breeding pairs of B6.Cg-*Msr1*^{tm1Csk}/J (SRA$^{-/-}$) mice (catalog no. 006096, Jackson Laboratories) were purchased to generate a total of 64 female SRA$^{-/-}$ mice for the experiments. Male mice were not used in experimental groups to decrease the risk of having to alter group numbers due to fighting. All mice were free of infection with mouse hepatitis virus, minute virus of mice, mouse parvovirus, mouse norovirus, Theiler murine encephalomyelitis virus, epizootic diarrhea of infant mice virus, Sendai virus, *M. pulmonis*, pneumonia virus of mice, reovirus 3, lymphocytic choriomeningitis virus, ectromelia virus, and endo- and ectoparasites, as monitored by testing of dirty-bedding sentinel mice housed in the same environment.

Equipment and housing. Mice were housed in groups of 4 on Tek-Fresh bedding (Harlan Teklad, Indianapolis, IN) in Max 75 polycarbonate cages (Alternative Design Manufacturing and Supply, Siloam Springs, AR). Mice received standard rodent chow (2018 Teklad Global 18% Protein Diet, Harlan Teklad) and tap water ad libitum. Mice were divided into groups for housing under filter tops without forced ventilation (hereafter referred to as static cages) or in IVC connected to a ventilation rack (Alternative Design Manufacturing and Supply) providing positive pressure at 50 air changes hourly. The bedding of each cage was measured to be approximately 1 cm deep. A gas sampling port was installed in the front of the cage by using a stainless steel commercial bulkhead barbed tubing connection (part no. MBHA-1332-316, Beswick, Greenland, NH). The bottom of the port was approximately 1.9 cm above the top of the bedding, the approximate height of a mouse's nose. A 4-cm piece of plastic intravenous tubing (catalog no. 2C5630, Baxter Healthcare, Deerfield, IL) was attached to the exterior of the sampling port. The luer-lock cap and standard pinch clamp on the tubing were used to effectively stop air from flowing in and out of the port during air sampling. The Drager CMS Gas Analyzer and Remote System (model nos. 6405300 and

6405060, Industrial Environmental, Worthington, OH) were used for NH_3 gas measurements. The gas-analysis system is electronic and self-calibrating. The system performs a self-test when it is turned on and calibrates when the measurement chip is inserted into the machine. All breeding mice were housed in a barrier facility that maintains mice in IVC with corncob bedding. When the pups reached weaning age, they were moved into the experimental room, and cages were changed at 14-d intervals by using Tek-Fresh bedding (Harlan Teklad), which is used by some investigators in our facility. Tek-Fresh is a wood-pulp–based bedding that does not control NH_3 production as well as does corncob bedding.[92] Therefore, to analyze the effects of increased intracage NH_3 levels, Tek-Fresh was our experimental bedding of choice. Although the *Guide* recommends that static cages be changed once weekly, it does allow for institutions to use outcome-based performance standards that ensure that animal health and welfare are not compromised. The current practice at our institution is to change cages every 14 d, with the exception of special cases that require more frequent changes.

Study design. Animal care staff randomly allocated mice into groups of 4, and all mice were housed in IVC with Tek-fresh bedding (Harlan Teklad) and 50 air changes hourly during acclimation. Once acclimated, mice were assigned randomly to either static or ventilated cages. In experiment 1, mice (designated as 'no vehicle') had 14-d cage change intervals. These mice were used to obtain baseline cell counts after bronchoalveolar lavage (BAL). They did not receive any intranasal inoculations prior to euthanasia and BAL collection. In the mycoplasma experiments, WT and $SRA^{-/-}$ mice, ranging from 11 to 16 wk in age, were infected with 10^7 cfu of *M. pulmonis*. These cages were changed every 14 d, as is standard at our institution. There were at least 2 cage changes prior to infection with *M. pulmonis*. Mice were euthanized at 3, 7, or 14 d after inoculation.

NH_3 measurements. For intracage NH_3 sampling, the plastic tubing was removed from the port and the sampling hose was attached. The hose was allowed to flush for 6 min prior to each sampling. Samples were recorded daily between 1500 and 1700. For NH_3 measurements, chips with a detection range of 2.0 to 50 ppm and 10 to 150 ppm were used. Chips were accurate to 7% to 8% of the measured value and reproducible at 10% to 12%.[24,25]

Infection of mice with *M. pulmonis*. Mice were infected intranasally with the UAB CT strain of *M. pulmonis*.[73] Stock cultures of *M. pulmonis* were grown in mycoplasma broth: mycoplasma broth base, 20 g; 1.0% phenol red, 2 mL; inactivated horse serum, 200 mL; 50% glucose, 10 mL; IsoVitalex (catalog no. 211875, Becton Dickinson, Franklin Lakes, NJ), 5 mL; DNA 20% stock, 1 mg; cefoperazone sodium salt 250 mg/mL (catalog no. C4292, Sigma–Aldrich, St Louis, MO), 260 µL. The medium was adjusted to pH 7.8; 1 mL of stock culture was added to 9 mL of the mycoplasma broth and incubated at 37 °C with shaking at 50 rpm. The organisms were harvested at late-log phase. Numbers of *M. pulmonis* bacteria (cfu) were determined by performing eight 10-fold serial dilutions on mycoplasma agar plates that were freshly prepared by using 1% SeaPlaque agarose (catalog no. NC9664839, Fisher Scientific, Waltham, MA). Plates were incubated at 37 °C and colonies were counted after 10 to 14 d by using a colony counter (model 920A, American Bantex, Burlingame, CA). *M. pulmonis* stock cultures were stored frozen in 1-mL aliquots at −80 °C. On the day of infections, aliquots were thawed on ice, added to 9 mL mycoplasma broth, and grown for 3 h in a 37 °C incubator with shaking at 150 rpm. The culture then was centrifuged at 3210 × *g* for 22 min. The supernatant was discarded and the pellet was resuspended with 5 mL of high-glucose DMEM (catalog no. 11965-084, Life Technologies, Grand Island, NY). The sample was centrifuged again at 3210 × *g* for 22 min at 4 °C. Cell pellets were resuspended in high-glucose DMEM and used for infections. Prior to infections, mice were anesthetized with ketamine (100 mg/kg IP) and xylazine (10 mg/kg). Mice then were intranasally inoculated with 30 µL of medium containing 10^7 cfu of *M. pulmonis*. Mice were euthanized for analysis at 3, 7, and 14 d after inoculation. For quantitation of *M. pulmonis* in the lungs, the lungs were homogenized in 2.5 mL PBS and plated as described earlier. The control (vehicle) group received 30 µL high-glucose DMEM intranasally and was euthanized at 3 d after inoculation. Mice did not display any respiratory difficulties throughout the study.

BAL. Anesthetized mice were euthanized by exsanguination after laceration of the abdominal aorta. The mice from the no-vehicle group were studied at the end of 2 consecutive cage-change cycles. Mice from the vehicle-control group were analyzed 3 d after the administration of sterile DMEM. Mice from the infection groups were euthanized at 3, 7, or 14 d after inoculation. BAL fluid was collected from 6 mice in each group. BAL fluid was obtained by repeatedly instilling PBS containing 1 mM EDTA into the lungs through a tracheal cannula in a volume equal to 80% of lung vital capacity (0.5 mL), for a total of 2.5 mL. For each of the 5 washes, the fluid was instilled and withdrawn 3 times with chest massage during withdrawal. The BAL fluid was centrifuged at 100 × *g* for 6 min and the cell pellet washed with 0.50 mL of PBS–EDTA. Total cell counts were obtained by using a hemocytometer, and cytocentrifuge preparations were generated for differential cell counts. The washed cells were centrifuged again, and the pellets were frozen in Trizol reagent (catalog no. 15596018, Life Technologies) at −80 °C for subsequent studies. Lungs were homogenized, aliquoted into 50-µL samples, and frozen at −80 °C for subsequent studies. Lavage and lung homogenate samples from each mouse were used to inoculate plates to obtain a mycoplasma count (no. of cfu).

Histopathology. The lungs were fixed by injecting 1 mL 10% neutral buffered formalin through a 27-gauge needle and into the trachea. The lungs then were removed and allowed to fix in 10% neutral buffered formalin overnight. The pelt was removed from the skull, and the head was decalcified in Formical-4 (Decal Chemical, Tallman, NY). Tissues were processed in an automated processor (Tissue-Tek VIP, Sakura Finetek USA, Torrance, CA) and paraffin-embedded (Tissue-Tek TEC, Sakura Finetek USA). Sections were cut at 6 µm for routine hematoxylin and eosin staining. Two mice from each group were evaluated by histology. All images were obtained by using an Olympus BX51 microscope, DP71 digital camera, and CellSens Standard 1.6 imaging software (all from Olympus America, Center Valley, PA).

Protein assay. The Pierce Micro BCA Protein Assay (Thermo Fisher Scientific, Hudson, NH) was used to determine the amount of protein in BAL fluids (25 µL in deionized H_2O). Micro BCA Reagents were mixed according to the manufacturer's instructions. RNase A was used as a standard. Absorbance was measured by using a plate reader at 570 nm.

Gel electrophoresis and Western blotting. Aliquots of BAL fluid (200 µL) were frozen at −80 °C, lyophilized to complete dryness, and resuspended in 40 µL of reducing SDS–PAGE Laemmli sample buffer (for SPA and SPD proteins) or nonreducing sample buffer (for SPB). Sample application strips (80-1129-46, GE Healthcare, Waukesha, WI) were applied to discontinuous 12.5% ExcelGel

SDS–polyacrylamide gels (GE Healthcare) and loaded with 5 µL BAL protein. Proteins were electophoretically separated and electrotransferred to a nitrocellulose membrane on a horizontal electrophoresis and blotting system (Multiphor II and NovaBlot unit, Pharmacia Biotech, Piscataway, NJ) by using a computer-controlled power supply (model 3000xi, BioRad, Hercules, CA). Electrophoresis was performed at 300 V and 20 mA for 30 min, 300 V and 50 mA for 15 min, and then for an additional 20 min after removal of sample application strips. Proteins then were transferred to nitrocellulose membrane at 12 V and 250 mA for 90 min. The membranes were blocked in blocking solution (1% BSA in PBS) for 1 h, washed (0.05% Tween 20 in PBS), and incubated at room temperature with rabbit antiSPA,[99] SPD, or SPB antibody (diluted 1:10,000 in PBS supplemented with 1% BSA and 0.5% Tween 20; Southern Hills Bioreagents, Cincinnati, OH) for 1 h. The blots were rinsed, washed, and then incubated with the secondary antibody (dilution, 1:10,000; goat antirabbit IgG conjugated with horseradish peroxidase, catalog no. 170-6515, BioRad) for 1 h. The membrane was rinsed and washed in 0.05% Tween 20 in PBS and incubated with Western Lightning Plus Enhanced Chemiluminescence reagent mix (catalog no. NEL104001EA, Perkin Elmer, Waltham, MA) for 1 min. Blots were exposed to radiographic film to visualize surfactant proteins. Blots were scanned and analyzed (model GS800 Calibrated Densitometer and Quantity One software, BioRad). Data were plotted as the percentage change from the mean vehicle value.

Cytokine measurements. ELISA kits for mouse IL1β, TNFα, monocyte chemotactic protein 1 (MCP1), IL6 (Ready-Set-Go Kits, eBioscience, San Diego, CA), and keratinocyte-derived chemokine (CXCL1, KC; Quantikine ELISA Kit, R and D Systems, Minneapolis, MN) were used to assay BAL fluid and lung homogenate supernatant samples. ELISA were performed according to manufacturers' instructions.

Statistical analysis. Prism 6 software (GraphPad, La Jolla, CA) was used for all statistical analyses. All values reported as means ± SEM. Two-way ANOVA was used to compare parametric data from different groups of mice. The Tukey multiple comparison test was used for all analyses, except for the NH_3 concentration studies, for which Sidak multiple comparison test was used. In the case of colony counts, the Kruskal–Wallis test and Dunn posthoc test were used. A P value less than or equal to 0.05 was considered significant.

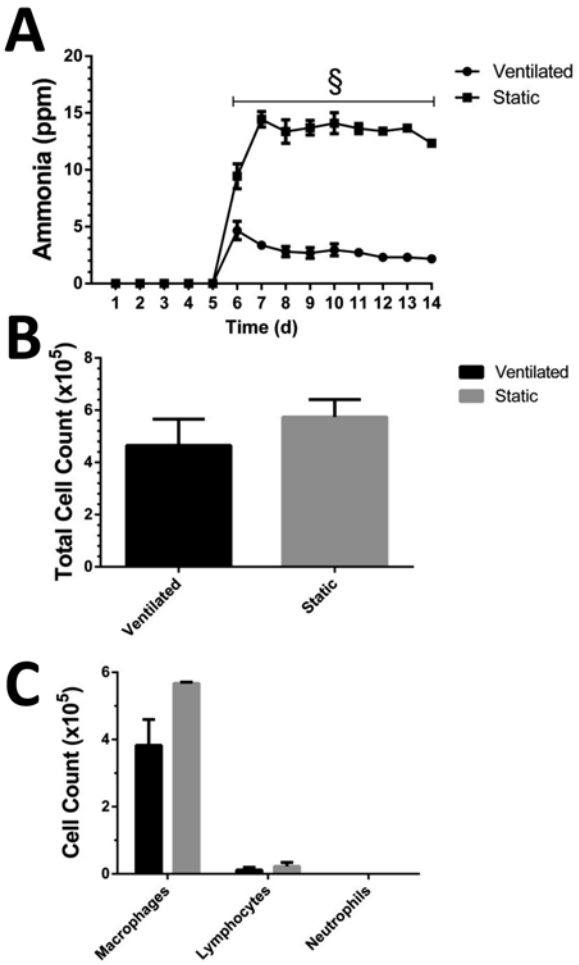

Figure 1. Accumulation of ammonia in ventilated and static cages. (A) Mice were placed in either static or ventilated cages at a density of 4 mice per cage and ammonia levels measured at the indicated times. Data are reported as the mean ± SEM of 3 cages measured at the same time. §, Difference between values is significant at $P \leq 0.0001$. The (B) total cell count and (C) cell differential were determined by cytospin analysis of BAL cells ($n = 6$). There were no significant differences between groups.

Results

NH_3 levels in IVC and static cages. Because SRA is involved in scavenging both pathogens[6] and particles,[110] we considered housing conditions as a variable in chronic *Mycoplasma pulmonis* infection. Typical husbandry conditions in our institution allow housing a maximum of 5 mice per cage and changing bedding every 2 wk within an SPF barrier facility. For infectious experiments, mice are transferred to a designated biosafety level 2 facility and usually are housed in static cages for the study duration. Ventilated cages were designed to prevent the accumulation of NH_3, which is produced by urease-positive bacteria from the gut microbiota, although previous studies showed that mice in ventilated cages experience increased levels of particulates as a consequence of forced air ventilation.[83] For the current study, we measured NH_3 levels as a surrogate assessment of the air quality in the mouse microenvironment in ventilated and static cages before infection in the BSL2 facility. Figure 1 A demonstrates that NH_3 levels on day 6 to 7 after residence were increased in 14-d cycled static compared with ventilated cages. The levels of NH_3 declined and subsequently stabilized at 2.1 ± 0.1 ppm in ventilated cages. In contrast, NH_3 continued to increase for another day before stabilizing more than 3-fold higher (12.3 ± 0.3 ppm) in static cages compared with ventilated cages. Higher levels of NH_3 in static cages did not cause obvious histopathologic changes in the nasal epithelium or lung. The total cell counts in BAL fluids were similar between mice in static and ventilated cages (Figure 1 B), although mice in static cages yielded consistently more alveolar macrophages (Figure 1 C). Alveolar macrophages were the predominant cell type under all conditions, with few lymphocytes (Figure 1 C). After confirmation of the differences in the ambient microenvironment between ventilated and static cage housed mice, subsequent experiments investigated *M. pulmonis* infection in 14-d cycled cages.

Effect of SRA on *M. pulmonis* infection in the lung. To examine the role of SRA in *M. pulmonis* infection, WT and SRA-deficient ($SRA^{-/-}$) mice were inoculated intranasally with 10^7 cfu *M. pulmonis* and euthanized at 3, 7, and 14 d after inoculation (Figure 2). WT mice in both static and ventilated caging had cleared 99.5%

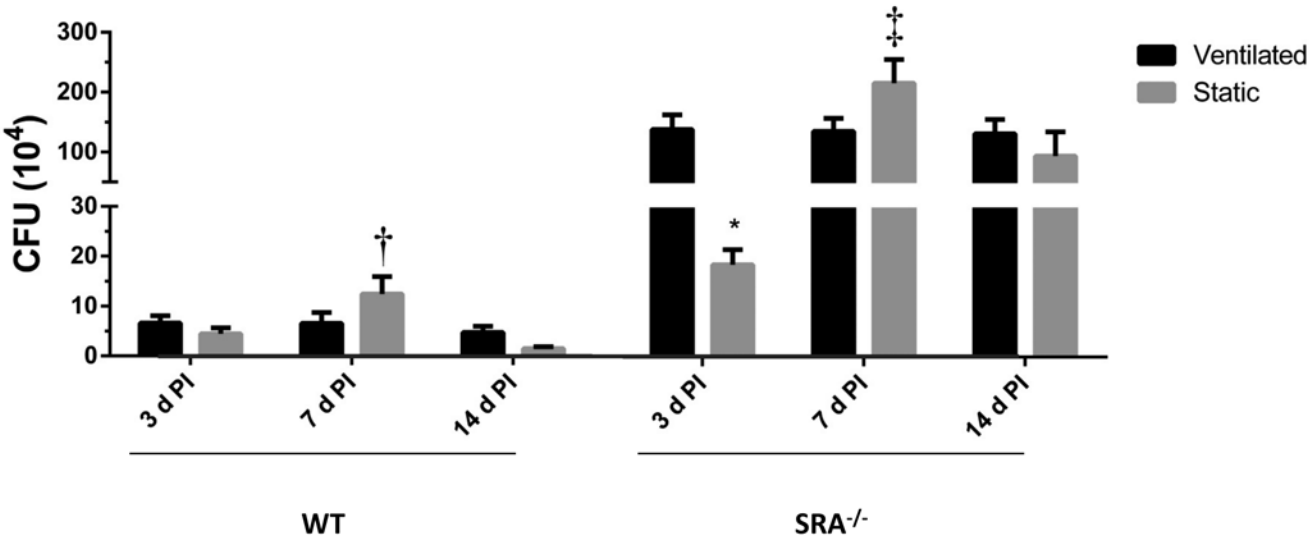

Figure 2. SRA suppresses *Mycoplasma pulmonis* infection in the lung. WT and SRA$^{-/-}$ mice housed in ventilated or static cages were infected intranasally with 10^7 cfu of *Mycoplasma pulmonis*. Lungs were harvested at 3, 7, and 14 after inoculation (PI), homogenized, and assayed for colony formation. Difference between values (mean ± SEM; $n = 6$ per group per time point) was significant (*, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$) compared to 3 d PI group of the same strain.

to 99.9% of the *M. pulmonis* inoculum by day 3, but remaining organisms led to persistent infection. Interestingly, *M. pulmonis* proliferated transiently in the lungs of WT mice housed in static cages but not in ventilated cages, as indicated by significantly ($P < 0.05$) increased colony counts between 3 and 7 d after inoculation and subsequent decline between 7 and 14 d (Figure 2). In contrast to WT mice, SRA$^{-/-}$ mice in ventilated caging had cleared only 80% of *M. pulmonis* infection at 3 d after inoculation, with no further reduction at 7 and 14 d (Figure 2). Compared with those in IVC, the SRA$^{-/-}$ mice in static cages had cleared significantly ($P < 0.05$) more (that is, 98.2%) of the inoculated dose of *M. pulmonis*, similar to the results in WT mice (Figure 2). However, this initial early clearance in the mice in static caging was only temporary, as indicated by the log-increase in proliferation of *M. pulmonis* by 7 d and persistence of infection at the same level as that in the ventilated group (Figure 2). These results indicate that lack of SRA diminishes innate and acquired immune responses against *M. pulmonis* infection in the lung, resulting in overall greater bacterial loads and slower resolution.

Effect of SRA on the inflammatory response to *M. pulmonis* pneumonia. Using differential staining, we assessed the effect of SRA deficiency and housing conditions on the influx of inflammatory cells into BAL fluid. WT mice in both ventilated and static caging showed only a mild transient increase in macrophages 3 d after *M. pulmonis* inoculation compared with the vehicle control, with only minimal influx of lymphocytic cells at days 7 and 14 d (Table 1). In contrast to the scenario in WT mice, the number of cells in the BAL fluid of SRA$^{-/-}$ mice remained 30-fold higher at both 3 and 7 d after inoculation in both ventilated and static caging groups and in the ventilated caging group at 14 d compared with vehicle controls (Figure 3 B). However, the number of cells in the BAL fluid of mice in static caging decreased by 3-fold compared with the counts of the ventilated caging group (Figure 3 B). Differential staining revealed that neutrophils were the major inflammatory cell type in the lungs of SRA$^{-/-}$ mice at 3 d after *M. pulmonis* inoculation in both the ventilated and static groups (Table 1). The inflammatory profile of SRA$^{-/-}$ mice was mixed at 7 and 14 d and consisted of macrophages, lymphocytes, plasma cells, and neutrophils (Table 1). The number of macrophages increased 4-fold and persisted at the same level for the duration of the experiment in mice in both ventilated and static caging. However, the influx of neutrophils had relented at 14 d after inoculation in the static group but remained persistently elevated in the ventilated group (Table 1).

The pathologic presentation of *M. pulmonis* pneumonia was assessed by using lung tissue sections stained with hematoxylin and eosin (Figure 4 and Table 1). The tissue morphology was normal in vehicle-treated WT and SRA$^{-/-}$ mice (Figure 4 A and B), except for a single vehicle-treated SRA$^{-/-}$ mouse in the static-caging group that showed mild inflammation. Overall findings from infected WT mice included mild peribronchiolar and perivascular lymphoid infiltrates and parenchymal consolidation, which decreased over time (Figure 4 C). In comparison, the SRA$^{-/-}$ mice had a more severe, progressive inflammatory response that was characterized by parenchymal consolidation, peribronchiolar and perivascular lymphocytic infiltrates, and neutrophilic exudates blocking the airways. By 14 d, overall inflammation in the lungs of SRA$^{-/-}$ mice housed in static caging appeared to be moving toward resolution. Neutrophilic exudates in this group were no longer statistically ($P < 0.05$) increased in the BAL fluid samples (Table 1) and were not present within the histologic sections of the airways (Figure 4 I). These results indicate that SRA controls the inflammatory response to *M. pulmonis* infection, although the inflammatory burden in the context of SRA deficiency appears to be influenced by the ambient microenvironment also.

Effect of SRA on inflammatory responses to *M. pulmonis* infection. To understand the role of SRA in the mixed inflammatory response to *M. pulmonis* and the potential role of the microenvironment in the intensity of this response, we measured the levels of key inflammatory cytokines (IL1β, TNFα, and IL6) and chemokines (KC and MCP1) in BAL fluids (Table 2) and lung homogenates (Figure 5).

We first measured IL1β as a marker of chronic inflammation. The IL1β concentration increased progressively in the BAL fluids

Table 1. BAL cell counts (x10^5, mean + SEM, n = 6 except for vehicle-control WT mice [n = 4])

		Macrophages	Lymphocytes	Plasma Cells	Neutrophils
Ventilated cages	WT				
	Vehicle	4.75 ± 0.00	0	0	0
	3 d PI	10.97 ± 0.00[c]	0	0	0
	7 d PI	3.25 ± 0.03	0.47 ± 0.03	0	0
	14 d PI	2.52 ± 0.05	0.08 ± 0.05	0	0
	SRA$^{-/-}$				
	Vehicle	5.11 ± 0.00	0	0	0
	3 d PI	20.03 ± 4.89	0	0	116.12 ± 27.97[c]
	7 d PI	23.36 ± 5.48	39.64 ± 9.21	59.34 ± 32.85	71.58 ± 28.55[a]
	14 d PI	32.04 ± 9.41	39.05 ± 9.77	72.69 ± 20.30[a]	127.77 ± 38.25[c]
Static cages	WT				
	Vehicle	5.13 ± 0.00	0	0	0
	3 d PI	8.50 ± 0.00[c]	0	0	0
	7 d PI	3.36 ± 0.01	0.60 ± 0.09	0	0
	14 d PI	2.09 ± 0.05	0.38 ± 0.10	0	0
	SRA$^{-/-}$				
	Vehicle	4.19 ± 0.00	0	0	0
	3 d PI	20.74 ± 4.69	6.74 ± 4.41	0	113.68 ± 28.60[c]
	7 d PI	17.54 ± 1.28	34.24 ± 15.88	24.16 ± 6.32	98.06 ± 45.65[b]
	14 d PI	15.56 ± 1.82	28.15 ± 10.50	28.22 ± 17.3	36.64 ± 25.05

PI, post inoculation with 10^7 cfu *Mycoplasma pulmonis*
[a]Value significantly ($P \leq 0.05$) different from that for the vehicle group of the same strain and housing conditions.
[b]Value significantly ($P \leq 0.001$) different from that for the vehicle group of the same strain and housing conditions.
[c]Value significantly ($P \leq 0.0001$) different from that for the vehicle group of the same strain and housing conditions.

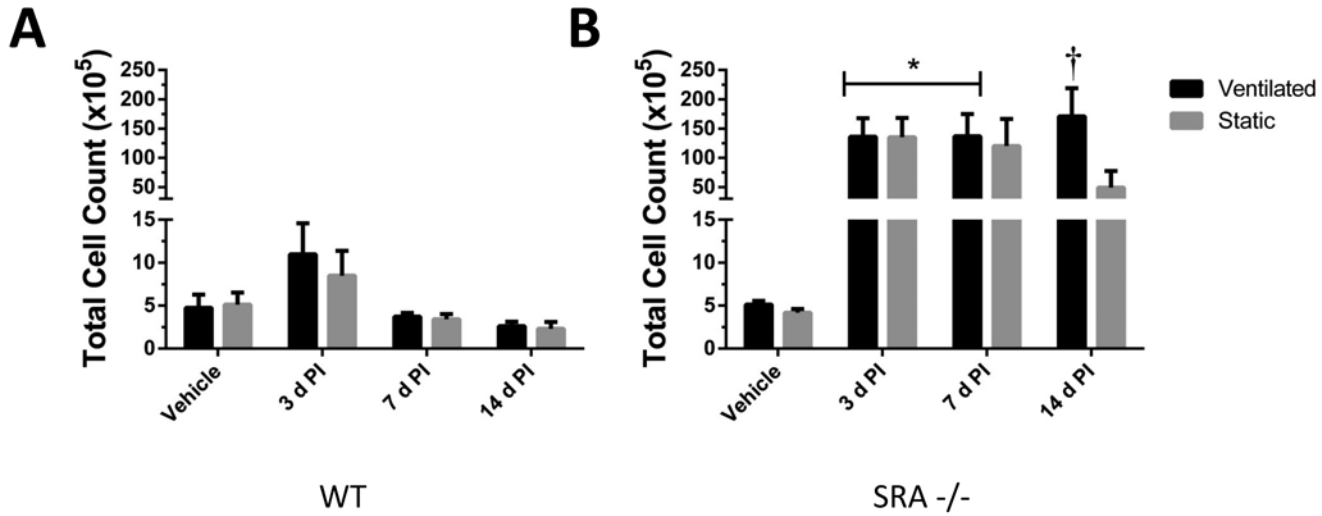

Figure 3. Pulmonary inflammation in *Mycoplasma pulmonis*-infected WT and SRA$^{-/-}$ mice. The total cell number were evaluated in lung lavage of (A) WT and (B) SRA$^{-/-}$ mice housed in either ventilated or static cages. The BAL was harvested 3, 7, and 14 d after inoculation with 10^7 cfu *Mycoplasma pulmonis* or 3 d after administration of vehicle. Value (mean ± SEM; n = 6 for all infected groups and SRA$^{-/-}$ vehicle mice; n = 4 for WT vehicle group) is significantly (*, $P \leq 0.05$; †, $P \leq 0.01$) different from that of respective vehicle group.

of both SRA$^{-/-}$ and WT mouse groups (Table 2). The levels of IL1β in the BAL fluids of SRA$^{-/-}$ mice reached a significant ($P < 0.05$) peak at 3 d after inoculation and remained greater ($P < 0.05$) than those in WT mice at 14 d after inoculation (Table 2). However, IL1β concentrations in WT and SRA$^{-/-}$ mouse lung homogenates did not change in response to inoculation of *M. pulmonis* compared with vehicle (Figure 5 A). In addition, endogenous IL1β levels were consistently and significantly ($P < 0.05$) lower in the lung homogenates of SRA$^{-/-}$ mice than WT mice at all time points (Figure 5 A).

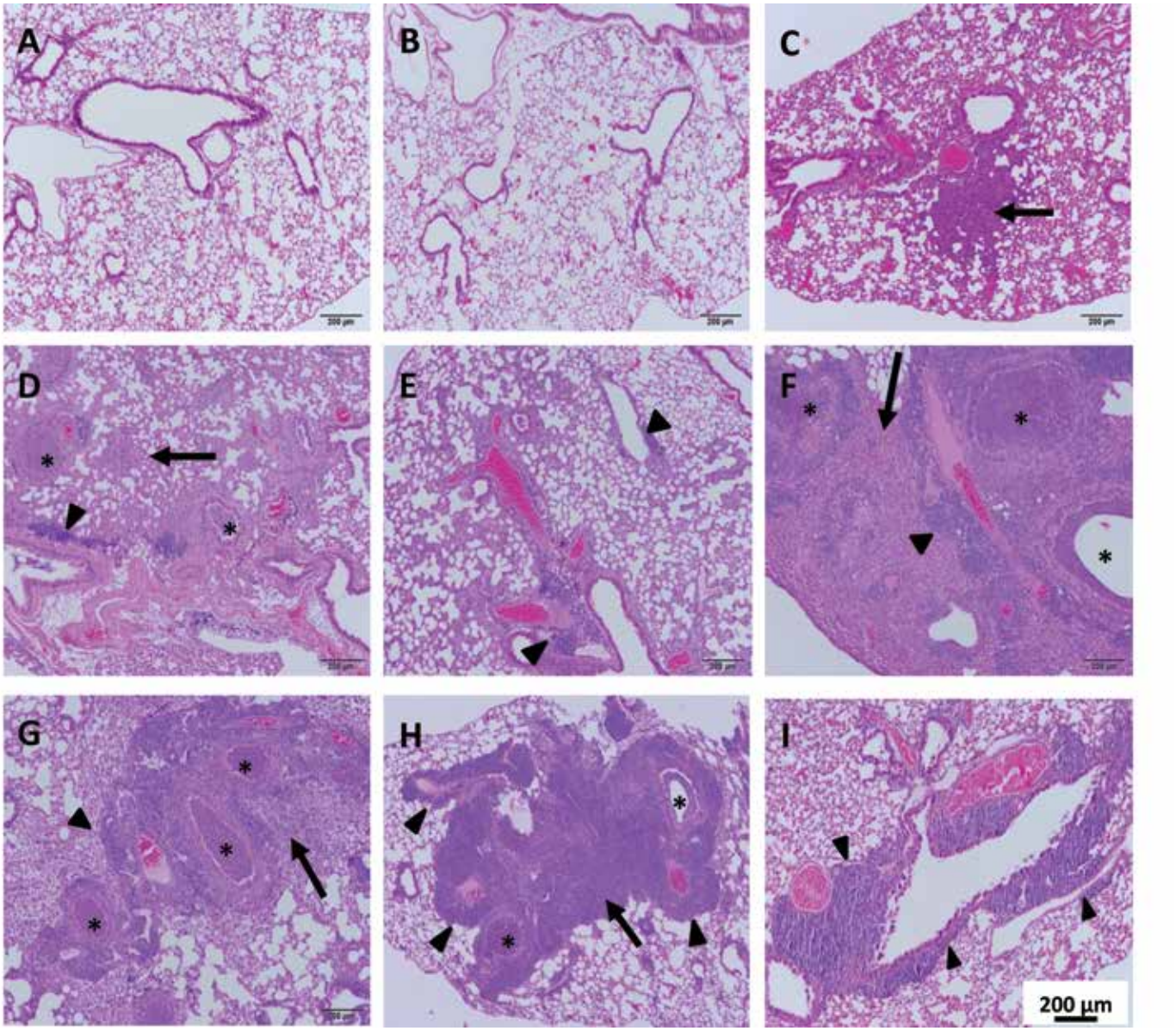

Figure 4. Lung histopathology of mice infected with *Mycoplasma pulmonis*. WT and SRA−/− mice were infected with 10^7 cfu of *Mycoplasma pulmonis*. Lungs were fixed by inflation and paraffin-embedded. Tissue sections were stained with hematoxylin and eosin and evaluated by light microscopy. Representative lungs of WT (A) and SRA (B) vehicle mice. (C) The lungs of an infected WT mice housed in the ventilated group had focal parenchymal inflammation at 3 d after inoculation. Infected SRA−/− mice housed in the (D, F, and H) ventilated group and (E, G, and I) static group developed parenchymal consolidation (arrow), perivascular–peribronchiolar lymphocytic cuffing (arrowhead), and neutrophilic exudates in the airways (asterisk) between 3 and 14 d after inoculation. Magnification, 10×.

The kinetics of TNFα, IL6, KC, and MCP1 in BAL fluids and lung homogenates differed between WT and $SRA^{-/-}$ mice. In contrast to levels in infected $SRA^{-/-}$ mice, TNFα, IL6, KC, and MCP1 were not detectable in the BAL fluids of any of the chronically infected WT mouse groups (Table 2). IL6 was undetectable in lung homogenates of WT mice at all 3 time points (Figure 5 C). However, IL6 levels in the BAL fluids of $SRA^{-/-}$ mice remained consistently increased between days 3 and 7 before declining at day 14 after inoculation (Table 2). Compared with those in WT mice with *M. pulmonis*, the levels of TNFα in the BAL of chronically infected $SRA^{-/-}$ mice were sharply elevated and reached significance ($P < 0.05$) at 3 d after inoculation (Table 2). Analysis of chemokines in BAL samples shows that KC was persistently elevated in $SRA^{-/-}$ mice throughout the course of the experiment (Table 2). The levels of MCP1 were significantly ($P < 0.05$) higher in $SRA^{-/-}$ mice than WT mice at days 3 and 7 after inoculation, after which the concentrations decreased gradually by day 14 after inoculation (Table 2).

The kinetics of TNFα (Figure 5 B), IL6 (Figure 5 C), and MCP1 (Figure 5 E) in $SRA^{-/-}$ lung homogenates paralleled those in BAL fluids (Table 2). The concentration of TNFα in lung homogenates was significantly ($P < 0.05$) elevated at 3 d for $SRA^{-/-}$

Table 2. Cytokine concentrations (pg/mL) in BAL of mice after inoculation (PI) with 10^7 CFU *Mycoplasma pulmonis* (infected) or sterile DMEM (vehicle)

	IL1β	TNFα	IL6	KC	MCP1
3 d PI vehicle					
WT ventilated cages	2.08 (0–2.08)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ ventilated cages	0.201 (0.07–0.27)	0.00	0.00	0.00	43.43 (29.38–57.47)
WT static cages	1.07 (0.31–1.82)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ static cages	0.00	0.00	0.00	0.00	33.51 (11.63–55.35)
3 d PI infected					
WT ventilated cages	2.66 (0.61–4.72)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ ventilated cages	40.62[a] (30.50–50.74)	232.19[c] (196.24–268.14)	298.93[c] (240.82–357.05)	91.77[b] (75.47–108.08)	372.10[c] (305.61–438.58)
WT static cages	16.91 (10.41–23.41)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ static cages	29.84 (23.84–36.00)	225.74[c] (156.58–294.90)	310.95[b] (199.39–422.51)	108.26[c] (84.88–131.65)	223.36[c] (180.77–265.95)
7 d PI infected					
WT ventilated cages	21.06 (18.53–23.60)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ ventilated cages	23.21 (15.87–30.52)	23.95 11.23–36.68)	211.37[b] (109.90–312.85)	66.75[a] (50.06–83.43)	217.03[b] (126.08–307.99)
WT static cages	31.31 (24.87–37.75)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ static cages	19.60 (10.16–29.05)	8.56 (2.52–14.61)	287.52[b] (164.85–410.18)	65.40[a] (43.08–87.71)	93.29[a] (47.86–138.73)
14 d PI infected					
WT ventilated cages	33.84 (32.12–35.56)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ ventilated cages	62.17[b] (48.53–75.82)	42.41 (28.73–56.10)	16.12 (4.83–27.40)	150.35[c] (105.88–194.82)	54.71 (37.71–71.71)
WT static cages	52.20 (9.75–63.64)	0.00	0.00	0.00	0.00
SRA$^{-/-}$ static cages	43.99 (36.37–51.61)	17.39 (5.66–29.11)	14.93 (4.67–25.18)	137.96[c] (103.95–171.96)	60.73 (45.71–75.75)

Data are shown as mean (95% confidence interval of the mean); $n = 6$ per group, except for WT vehicle group ($n = 4$)
[a]Value significantly ($P \leq 0.05$) different from that of the WT group of the same time point and housing conditions.
[b]Value significantly ($P \leq 0.01$) different from that of the WT group of the same time point and housing conditions.
[c]Value significantly ($P \leq 0.0001$) different from that of the WT group of the same time point and housing conditions.

mice in ventilated caging compared with WT mice (Figure 5 B). TNFα levels did not differ between WT and SRA$^{-/-}$ mice housed in static cages at any time point. The levels of lung homogenate KC (Figure 5 D) were significantly ($P < 0.05$) higher in IVC- and static-caging–housed SRA$^{-/-}$ mice compared with WT mice at 3 and 7 d after inoculation. Similarly, MCP1 levels in lung homogenate (Figure 5 E) were significantly ($P < 0.05$) elevated at days 3 and 7 after inoculation for SRA$^{-/-}$ mice compared with the WT mice. Both KC and MCP1 levels were highest at 3 d after inoculation (Figure 5 G through J) and then declined gradually by 14 d. Overall, lack of SRA drives a chronic and robust inflammatory response to *M. pulmonis* infection as indicated by high levels of multiple inflammatory mediators (Figure 3). There were no marked differences in the amounts of the inflammatory mediators tested (Table 2 and Figure 5) to explain the milder course of *M. pulmonis* pneumonia in the SRA$^{-/-}$ mice housed in static compared with ventilated caging (Figures 3 and 4).

Expression of surfactant proteins in WT and SRA$^{-/-}$ mice. Previous studies demonstrated that surfactant proteins SPA and SPD contribute to the inflammatory response to and clearance of mycoplasma pneumonia.[15,39,40] Therefore, we sought to determine whether both housing conditions and lack of SRA expression affect the levels of surfactant protein. We used Western blotting and densitometry to evaluate the levels of SPA and SPD. We also determined levels of SPB as a measure of surfactant integrity and assessed the effect of infection on protein concentration in BAL fluids (Figure 6). BAL protein levels in infected WT mice were stabilized at concentrations 2-fold higher than those in vehicle-control groups at 3 to 14 d after inoculation (Figure 6 A). In infected SRA$^{-/-}$ mice, however, BAL protein continuously increased over time and was 2- to 3-fold higher than that of control mice by 14 d after inoculation. Both ventilated and static groups of SRA$^{-/-}$ mice had significantly more protein than WT mice at days 7 and 14 after inoculation (Figure 6 A). Equal volumes of lavage fluid from WT and SRA$^{-/-}$ mice then were applied to SDS–PAGE to assess the levels of SPA (Figures 6 B and C, Figure 7), SPD (Figure 6 E and F, Figure 8), and SPB (Figure 6 F and G, Figure 9). The effects of mycoplasma infection on the levels of SPA and SPD levels differed between WT and SRA$^{-/-}$ mice. The type of housing also had a marked effect on SPA and SPD profiles. At 3 d after inoculation, the SPA level in the BAL fluid of WT mice in ventilated caging was significantly elevated relative to the control baseline (Figure 6 B). However, there were no signifi-

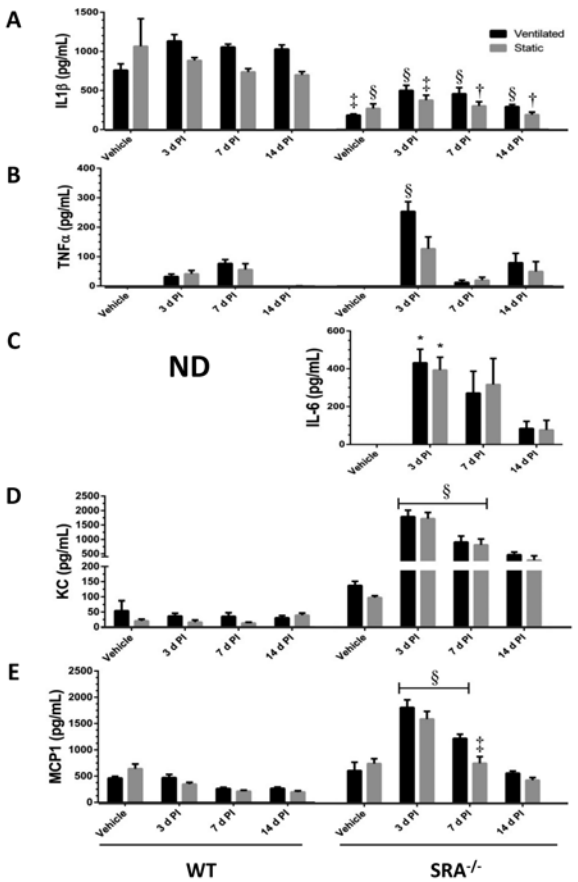

Figure 5. Lack of SRA enhances the levels of inflammatory mediators in lung tissue. Lavaged lungs of WT and SRA$^{-/-}$ mice harvested on days 3, 7, 14 after inoculation with 10^7 CFU of *Mycoplasma pulmonis* and vehicle-control mice were homogenized in PBS. The concentrations of IL1β, TNFα, IL6, KC, and MCP1 in the lung homogenates from mice housed in (black bars) ventilated and (grey bars) static caging were measured by ELISA. ND, not detected. Value (mean ± SEM; all SRA$^{-/-}$ groups and WT infected group, $n = 6$; WT vehicle group, $n = 4$) significantly (*, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$) different between WT and SRA$^{-/-}$ mice.

cant changes in SPA amounts among WT mice in static caging. In comparison, SRA$^{-/-}$ mice experienced a gradual and significant ($P < 0.05$) increase in SPA in the static-housing group at days 7 and 14 after inoculation (Figure 6 C).

SPD levels in WT mice in static caging were significantly ($P < 0.05$) decreased at 3 d after inoculation as a result of infection and then gradually increased toward baseline (Figure 6 D). The SRA$^{-/-}$ mice in both housing conditions had an overall immediate increase in SPD at 3 d after inoculation, but this change was significant ($P < 0.05$) only for the mice in static caging (Figure 6 E). SPB did not vary significantly from baseline in any of the WT or SRA$^{-/-}$ groups after mycoplasma infection (Figure 6 F and G). In SRA$^{-/-}$ mice housed in static cages, the higher levels of all 3 surfactant proteins at day 14 correlated with their milder *M. pulmonis* pneumonia. Taken together, these results indicate that environmental conditions can alter surfactant homeostasis in the context of SRA deficiency and contribute to the severity of chronic mycoplasmosis.

Discussion

Our study indicates that SRA enhances host resistance to mycoplasma pneumonia in a manner that depends on environmental conditions. That SRA is necessary to limit the severity of *M. pulmonis* infection is indicated by the persistence of more than 40-fold higher numbers of bacteria in the lung of SRA$^{-/-}$ compared with WT mice. SRA$^{-/-}$ mice developed a complex mixed inflammation with the involvement of both innate and adaptive immune cells, whereas WT (and thus resistant) mice experienced only mild monocytosis. The cytokine and chemokine profiles correlated with the cellular inflammatory response in SRA$^{-/-}$ and WT mice. An early and persistent infiltration and blockage of affected airways by neutrophils was a distinguishing feature of *M. pulmonis* pneumonia in SRA$^{-/-}$ but not WT mice. The subsequent appearance of plasma cells suggests an intense humoral immune response to *M. pulmonis* that may have helped stabilize the infection level in SRA$^{-/-}$ mice, although it was not sufficient to eliminate the infection. This outcome suggests that innate immunity is inadequate at clearing early *M. pulmonis* infection in the absence of SRA. In light of the role of SRA and other scavenger receptors in the clearance of environmental particulates and other contaminants as well as pathogens,[5,6] we considered whether the ambient microenvironments in ventilated and static cages influenced the course and severity of *M. pulmonis* pneumonia. We found that housing conditions did affect the pattern of chronic *M. pulmonis* infection in both WT and SRA$^{-/-}$ mice, even though the bacterial burden was 40- to 200-fold higher in SRA$^{-/-}$ than in WT mice. Furthermore, inflammatory profiles revealed that SRA$^{-/-}$ mice in static cages experienced less severe chronic inflammation. In this context, differences in surfactant protein levels may contribute to the severity and chronicity of *M. pulmonis* infection in WT and SRA$^{-/-}$ mice. Taken together, these findings emphasize the importance of SRA in immune homeostasis and genetic susceptibility to chronic mycoplasmosis.

Phagocytosis is critical for the killing of mycoplasma by macrophages.[42,52] Furthermore, depletion of alveolar macrophages has been shown to significantly enhance susceptibility to *M. pulmonis*, indicating that alveolar macrophages are essential for mycoplasma clearance.[42] The high level of *M. pulmonis* infection in the lungs of SRA$^{-/-}$ mice compared with WT mice might be attributed to decreased recognition of the organism by SRA on macrophages and its subsequent killing by these cells. Lack of SRA impairs macrophage binding to and phagocytosis of *Streptococcus pneumoniae*,[6] *Staphylococcus aureus*,[88] *Escherichia coli*,[73] and *Haemophilus influenzae*.[33] However, surfactant proteins SPA and SPD as well as the surfactant phospholipid phosphatidyl glycerol have been shown to attenuate the growth of *M. pulmonis* and *M. pneumoniae* as well as opsonize and enhance the clearance of the organisms from the lung.[15,39,40,43,46,74] SPA-mediated uptake of *M. pulmonis* enhanced activation of alveolar macrophages via the production of nitric oxide and peroxynitrite radicals.[39,40,43] The binding of SPA to *M. pulmonis* may enhance clearance through coordinated interactions between the SPA receptor SPR210 and SRA.[88] In the cited study,[88] the lack of SRA was associated with decreased levels of SPR210 in alveolar macrophages and lung tissue. Therefore, one possible mechanism for the persistence of mycoplasma pneumonia is that the absence of SRA results in critical deficiency of surfactant-protein–mediated opsonization and clearance of *M. pulmonis.*

The infection of SRA$^{-/-}$ mice with *M. pulmonis* is associated with a broad increase in inflammatory mediators and immune

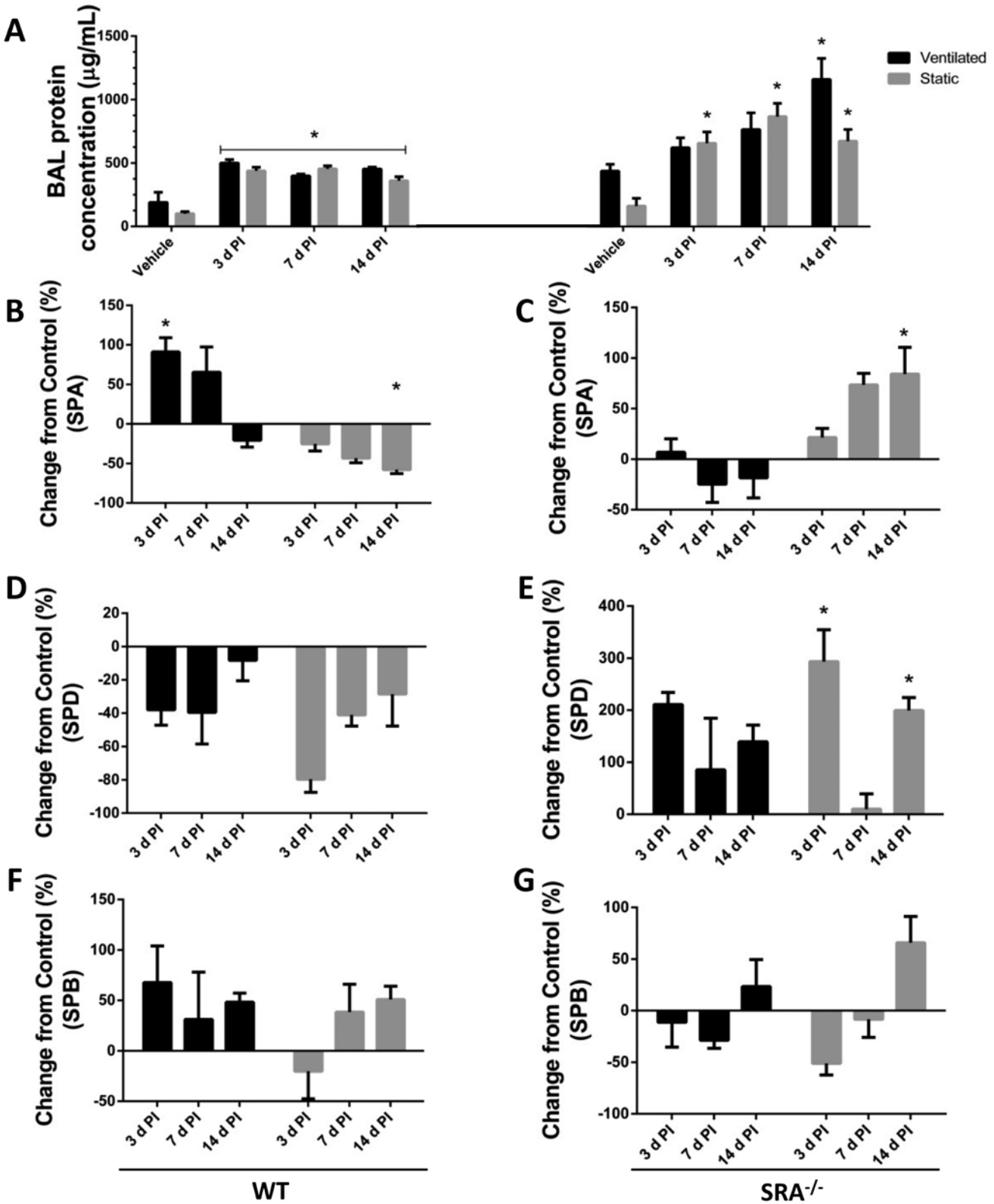

Figure 6. Protein levels in the lavage of WT and SRA$^{-/-}$ mice. Lavage was collected from vehicle-treated or *Mycoplasma pulmonis*-infected WT or SRA$^{-/-}$ mice at the indicated time points. Samples were analyzed for (A) protein content by using the BCA assay and for the individual surfactant proteins (B and C) SPA, (D and E) SPD, and (F and G) SPB by Western blotting and densitometry. Percentage change (mean ± SEM; all SRA$^{-/-}$ groups and WT infected group, $n = 6$; WT vehicle group, $n = 4$) from the mean value of the vehicle-control groups was plotted. Value significantly (*, $P \leq 0.05$) different from baseline value.

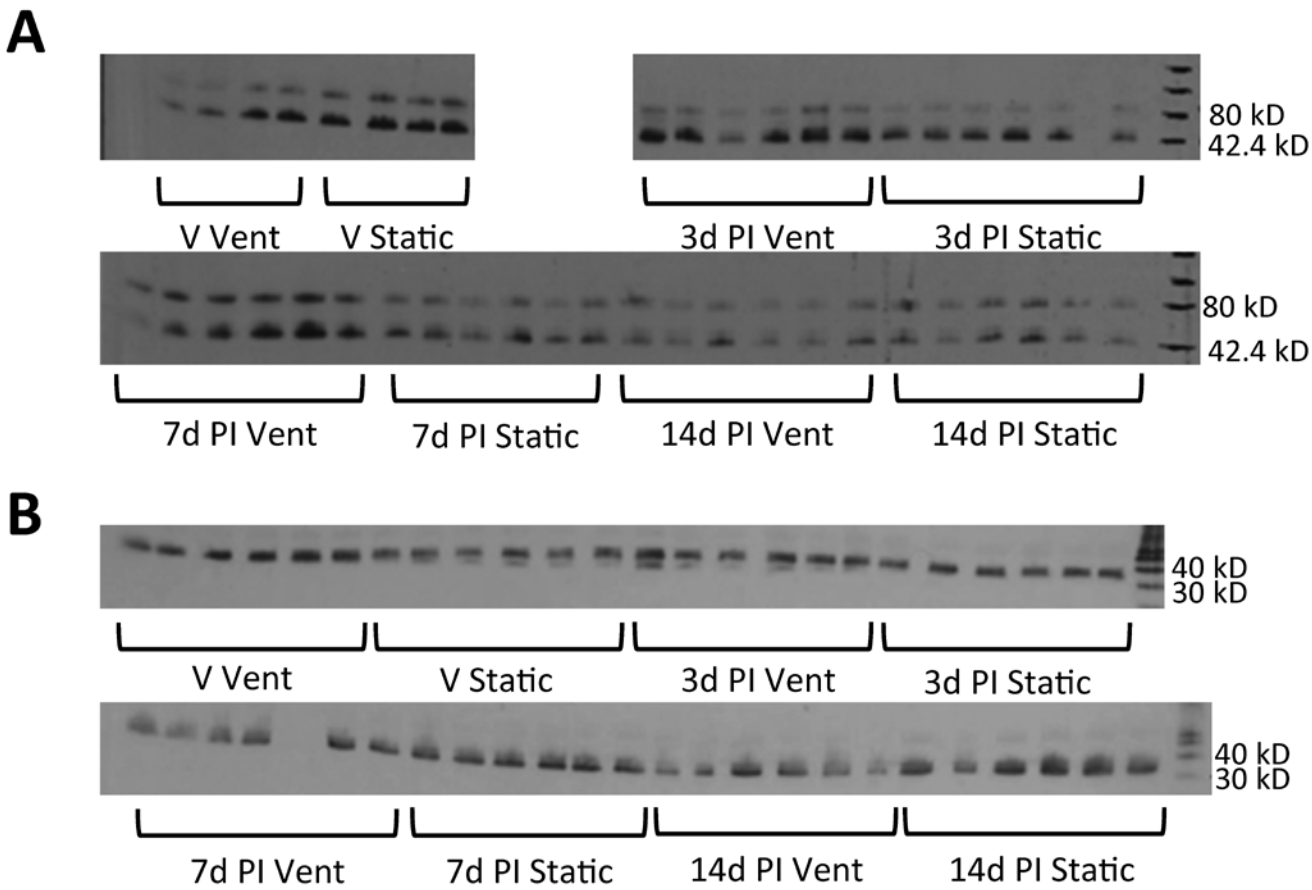

Figure 7. Corresponding Western blots of BAL from (A) WT and (B) SRA$^{-/-}$ mice show SPA as an approximately 36-kDa monomer or approximately 64-kDa dimer. V, vehicle; Vent, ventilated caging; Static, static caging; PI, postinoculation.

cells. Previous studies showed that TLR2 mediates the inflammatory response to mycoplasma infection,[46,60,104] thus TLR2 and other TLR receptor agonists enhance the phagocytic activity of SRA.[2,106] Therefore, lack of SRA may have resulted in functional uncoupling between the inflammatory response and clearance pathway of *M. pulmonis* via TLR2 and SRA, respectively. The enhanced and persistent production of IL6 indicates the activation of TLR2 in SRA$^{-/-}$ mice. In contrast, other studies show that SRA regulates the behavior and activation state of mononuclear phagocytes, as indicated by changes in alveolar macrophage phenotype and increased mobility and maturation of dendritic cells.[5,88]

Plasma cell counts were highly elevated in the lavage fluids of *M. pulmonis*-infected SRA$^{-/-}$ mice, suggesting that B lymphocyte differentiation was accelerated also. In this regard, SRA expression in dendritic cells facilitates the acquisition of antigen from B lymphocytes in lymph nodes.[36,37] One possibility in our mice is that SRA$^{-/-}$ dendritic cells migrated out of the lung mucosa prematurely, having sampled antigen at the early but not late stage of infection and subsequently resulting in inadequate humoral and cell-mediated immunity. The persistence of neutrophils may be a potential source of tissue damage that could enhance attachment and chronic colonization with *M. pulmonis*. KC is a key neutrophil chemoattractant, causing neutrophilic infiltration of the airways, which is a common feature of mycoplasmosis.[1,109] Tissue damage initiated by neutrophils may propagate the inflammatory response via the release of endogenous mediators. Furthermore, TNFα and MCP1 levels increased early and persisted in infected SRA$^{-/-}$ mice. Although these inflammatory mediators enhance the activation and recruitment of immune cells, they also act on endothelial and epithelial cells. TNFα drives remodeling of capillary and lymphatic vessels,[7,27] and MCP1 enhances expression of epithelial mucins.[67] Taken together, these results indicate that SRA is needed not only to facilitate clearance of infection but also to coordinate appropriate levels of inflammation and host defense to *M. pulmonis* infection.

The secretion of IL1β may serve as a biomarker for chronic infection, as indicated by the increased levels of IL1β in the BAL fluids of *M. pulmonis*-infected mice. Recent studies showed that IL1β is essential for resistance to *Chlamydophila pneumoniae* infection.[89] Lack of caspase 1 impaired TLR2-mediated secretion of IL1β, resulting in decreased nitric oxide production and clearance of the infection by alveolar macrophages as well as increased inflammation in the lung. IL1β is synthesized as a biologically inactive proIL1β form.[107] Activation of the NLRP3 inflammasome in the cytosol results in activation of caspase 1, which cleaves proIL1β into biologically active IL1β. Mature IL1β then is secreted via an unconventional secretory pathway.[4,30,69,70] The ELISA that we used in the current study does not distinguish proIL1β from IL1β. However, the increased IL1β levels in BAL samples from *M. pulmonis*-infected WT and SRA$^{-/-}$ mice are

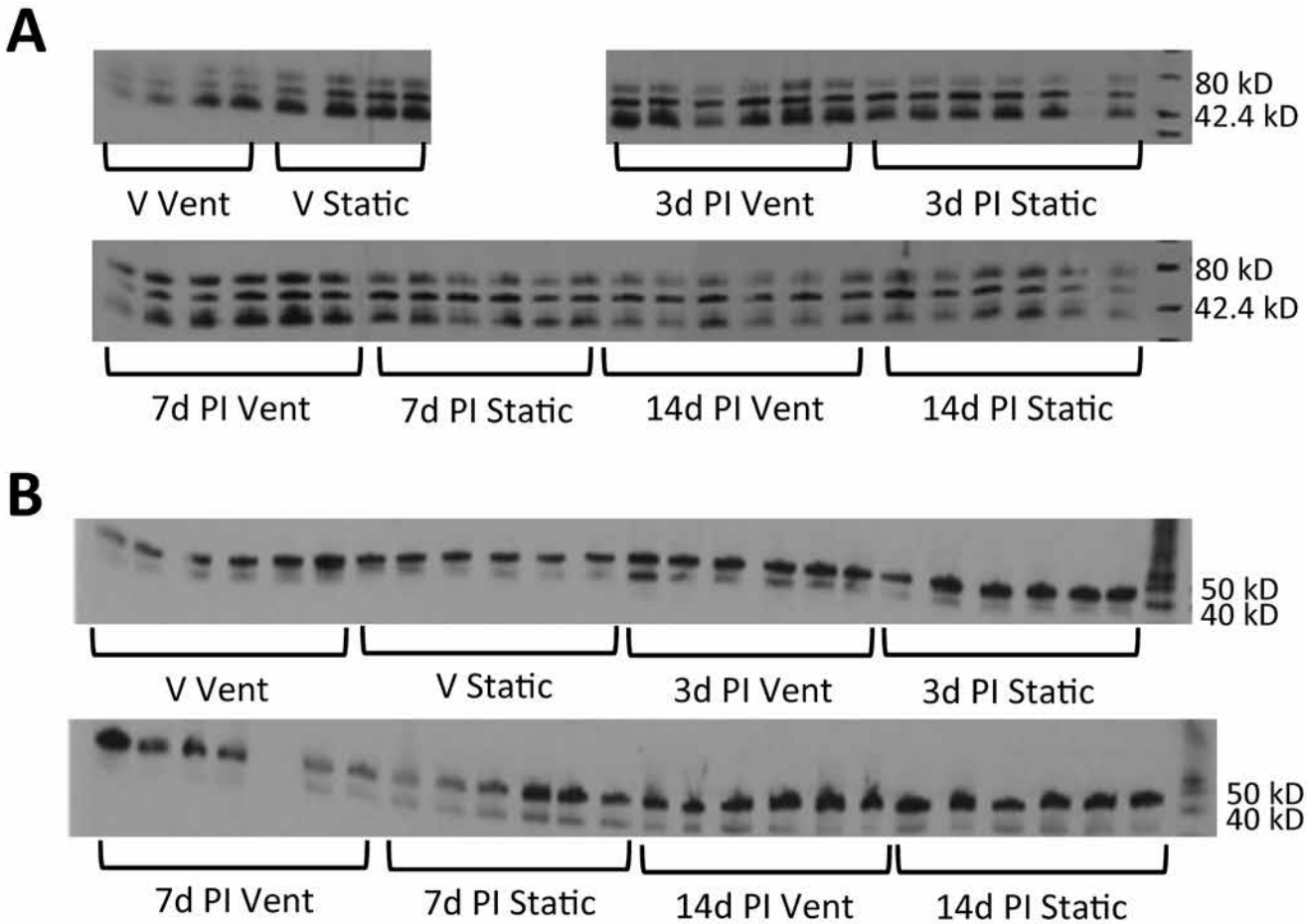

Figure 8. Corresponding Western blots of BAL from (A) WT and (B) SRA$^{-/-}$ mice show SPD as a 40- to 50-kDa monomer. Spurious bands above and below the SPD signal are artifacts from SPA proteins. V, vehicle; Vent, ventilated caging; Static, static caging; PI, postinoculation.

consistent with ongoing secretion of active IL1β. Previous studies[9] reported that the expression of proIL1β is either induced by inflammatory stimuli or constitutively expressed in different cell types. Neutrophils and monocytic cells in the lung have been shown to constitutively express proIL1β.[9] We found high levels of total IL1β in lung homogenates from both infected and uninfected WT and SRA$^{-/-}$ mice (Figure 5 A), although we did not determine the levels of active IL1β compared with proIL1β in the present study. However, the levels of IL1β were lower in SRA$^{-/-}$ mice than in WT mice. SRA has been described as a cell-surface sensor for the activation of IL1β.[34,50] IL1β drives the recruitment and activation of macrophages.[80] Therefore, a lack of SRA may alter the balance of myeloid cells toward neutrophil-driven inflammation in SRA$^{-/-}$ mice. Taken together, these results support the notion that SRA plays a role in maintaining optimal levels of proIL1β in the lung.

Studies in mice with different genetic backgrounds have demonstrated marked variability in susceptibility to mycoplasma infection. BALB/c and C3H mice are highly susceptible to *Mycoplasma*, whereas C57BL/6 mice are highly resistant.[14,21,93] The SRA genes of BALB/c and C3H mice contain polymorphisms,[19,29] although the functional significance of this finding has not been established. The lungs of susceptible BALB/c mice expressed high levels of IL1β, IL6, TNFα, and KC in response to *M. pneumoniae* infection.[28] Similar to the scenario in SRA$^{-/-}$ mice, the disruption of surfactant proteins SPA and SPD in C57BL/6 mice leads to increased inflammation and enhances susceptibility to *M. pulmonis* and *M. pneumoniae*.[15,39,43,54,55,74,98] Common polymorphisms in the SPA gene contribute to increased risk for development of COPD[35,61,86] and asthma.[100] Therefore, the concurrent presence of polymorphisms in the genes for SRA and surfactant proteins may enhance susceptibility to chronic infection and inflammation in the lung.

Our current results also indicate that housing conditions affect immune responses to mycoplasma infection in the context of SRA deficiency. We found that mice housed in static cages experienced less severe inflammation and differences in the pattern of chronic infection compared with mice in ventilated cages. We anticipated that mice would fare worse in static cages, given the accumulation of ambient NH_3. NH_3 levels can reach as high as 700 ppm in static cages housing mice.[90] However the NH_3 levels in the present study did not reach 25 ppm, which is generally considered a threshold of toxicity.[95] Differences in animal density, bedding, gas exchange through the filtered lid, macroenvironment, and urinary output between mouse strains can influence the level of NH_3 accumulation.[64,77,81,83,90] In the present study, mice in static cages had cleared more mycoplasma at 3 d, but the organism proliferated between days 3 and 7 before establishing chronic infection. In comparison, mice in IVC reached an infection plateau by 3 d that remained consistent through

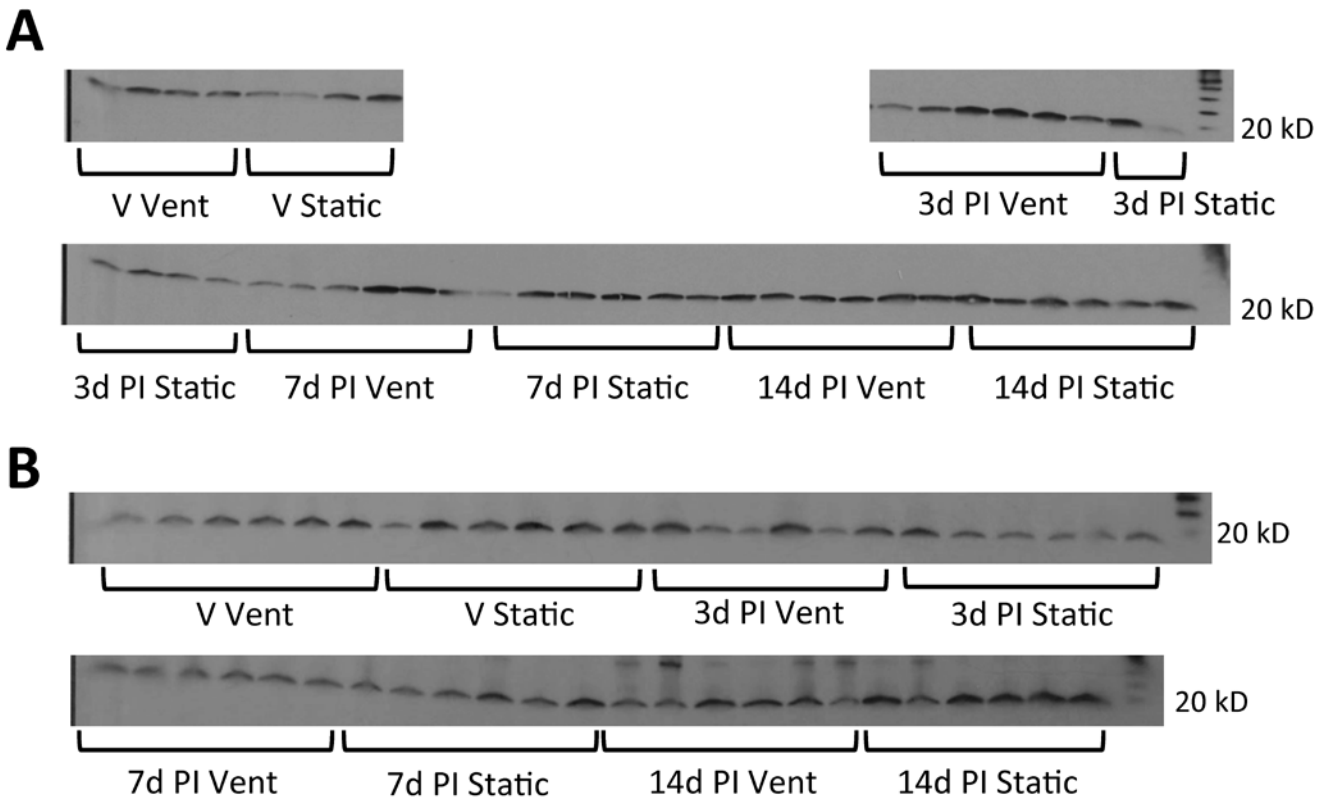

Figure 9. Corresponding Western blots of BAL from (A) WT and (B) SRA$^{-/-}$ mice show SPB as an approximately 18-kDa band. V, vehicle; Vent, ventilated caging; Static, static caging; PI, postinoculation.

day 14. This pattern of infection correlated with the kinetics of NH_3 accumulation in static cages, suggesting that NH_3 played a role. Histologic assessment of a larger group of mice is necessary to statistically support our initial findings on the effects of microenvironment and chronic mycoplasmosis in SRA$^{-/-}$ mice. The use of ventilated caging in research facilities was introduced to reduce NH_3 accumulation, cage change frequency, support housing of more mice per cage, and limit exposure of laboratory personnel to airborne allergens.[78,81,82,90,91] However, mice in ventilated cages may experience decreased humidity and increased levels of airborne particulates and dust from bedding as consequences of forced air ventilation and animal activity in the cages.[83] The macroenvironment in the animal facility may contribute to increased levels of intracage microparticulates.[83] Relevant to these findings, occupational exposure to dust particles contributes to the development of respiratory ailments in both animals and humans.[53,75,76,84] These particles may inhibit the binding of *M. pulmonis* to macrophages and thus contribute to chronic infection in WT mice. Additional studies are needed to determine whether intracage 'air pollution' and the lack of SRA scavenger function prime the lung of SRA$^{-/-}$ mice for chronic inflammation.

Few suitable models are available to understand the relationship between chronic respiratory infection and environmental factors. In the present model, we show that absence of SRA causes chronic clinical mycoplasmosis in mice. Infection of SRA$^{-/-}$ mice with *M. pulmonis* results in persistent suppurative bronchopneumonia, with airway obstruction, bronchiectasis, activation of bronchus-associated lymphoid tissue, and chronic inflammation. SRA may contribute to immune homeostasis by fine-tuning the levels of surfactant proteins and IL1β in myeloid cells in the lung. Differences in ambient air quality as generated by different housing conditions affect the pattern of *M. pulmonis* infection, resulting in either temporal exacerbation of inflammation or stable chronic infection. Researchers and animal care facilities must be aware that inter- and intrainstitutional differences in animal husbandry and housing practices may alter the physiologic and phenotypic outcome of mice, yielding variable results.

Acknowledgments

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